TECHNICAL BULLETIN

pGEM®-9Zf(–) Vector

Instructions for Use of Product
P2391

Revised 4/17
TB070

pGEM®-9Zf(–) Vector

All technical literature is available at: www.promega.com/protocols/
Visit the web site to verify that you are using the most current version of this Technical Bulletin.
E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

1. Description

The pGEM®-9Zf(–) Vector is a recombinant plasmid designed to provide a versatile range of cloning strategies, and efficient synthesis of RNA in vitro. The plasmid contains T7 and SP6 RNA polymerase promoters flanking a multiple cloning region within the α-peptide coding region of β-galactosidase (1). Insertional inactivation of the α-peptide allows recombinant clones to be directly identified by color screening on indicator plates. The multiple cloning region is unique and includes restriction sites for NsiI, SpeI, HindIII, XbaI, EcoRI, SalI and SacI.

Promega vector sequences are available online at: **www.promega.com/vectors/** and are also available from the GenBank® database.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
pGEM®-9Zf(–) Vector	**20µg**	**P2391**

The pGEM®-9Zf(–) Vector is provided with a glycerol stock of bacterial strain JM109. The JM109 cells do not contain the vector and are not competent.

Storage Conditions: Store the pGEM®-9Zf(–) Vector at –20°C and the glycerol stock of JM109 cells at –70°C.

3. pGEM®-9Zf(–) Vector Multiple Cloning Region and Map

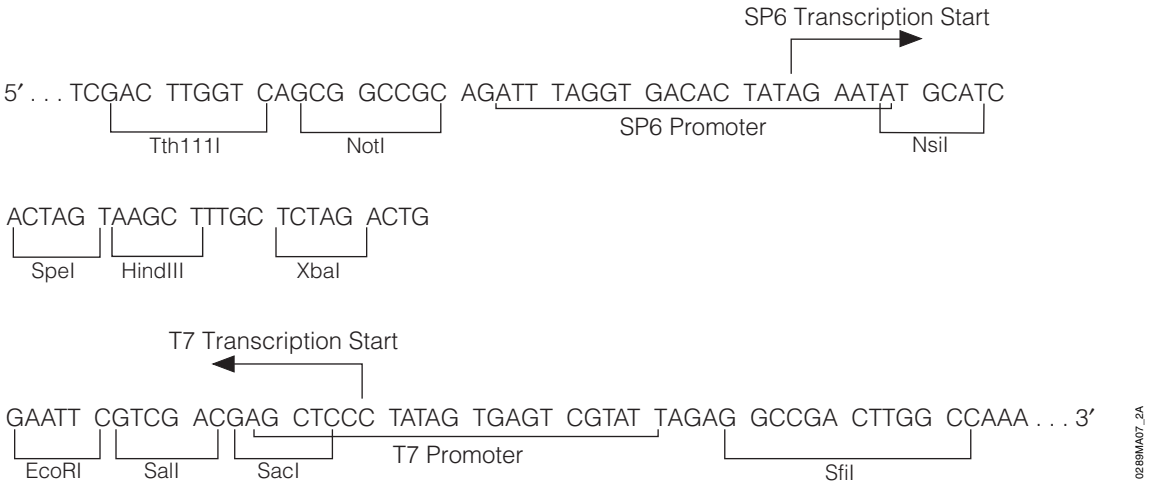

Figure 1. pGEM®-9Zf(–) Vector promoter and multiple cloning region sequence. The sequence shown corresponds to RNA synthesized by SP6 RNA polymerase and is complementary to RNA synthesized by T7 RNA polymerase.

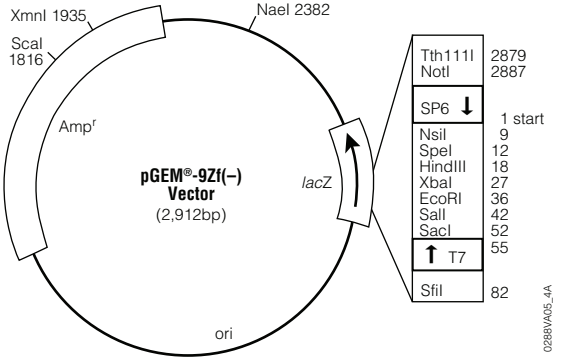

Figure 2. pGEM®-9Zf(–) Vector map and sequence reference points.

pGEM®-9Zf(–) Vector sequence reference points:

SP6 RNA polymerase transcription initiation site	1
T7 RNA polymerase promoter (–17 to +3)	53–72
T7 RNA polymerase transcription initiation site	55
lac operon sequences	93–321, 2712–2868
*lac*Z start codon	106
lac operator	126–142
β-lactamase (Amp^r) coding region	1263–2123
multiple cloning region	2876–87
SP6 RNA polymerase promoter (–17 to +3)	2896–3

Specialized applications of the pGEM®-9Zf(–) Vector:

- Allows excision of insert containing SP6 and T7 promoters
- Blue/white screening for recombinants
- Transcription in vitro from dual-opposed promoters (For protocol information please request the Riboprobe® in vitro Transcription Systems Technical Manual, #TM016.)

Promega

4. pGEM®-9Zf(−) Vector Restriction Sites

The following restriction enzyme tables were constructed using DNASTAR® sequence analysis software. Please note that we have not verified this information by restriction digestion with each enzyme listed. The location given specifies the 3´ end of the cut DNA (the base to the left of the cut site). For more information on the cut sites of these enzymes, or if you identify a discrepancy, please contact your local Promega Branch or Distributor. In the U.S., contact Promega Technical Services at 800-356-9526. Vector sequences are also available through GenBank® (GenBank®/EMBL Accession Number X65312) and on the Internet at: www.promega.com/vectors/

Table 1. Restriction Enzymes That Cut the pGEM®-9Zf(−) Vector Between 1 and 5 Times.

Enzyme	# of Sites	Location
AccI	1	43
AflIII	1	443
Alw26I	2	1397, 2173
Alw44I	2	757, 2003
AlwNI	1	859
AvaII	2	1474, 1696
BalI	1	85
BanI	3	187, 1284, 2444
BanII	2	52, 2414
BglI	3	82, 1456, 2723
BsaI	1	1397
BsaAI	1	2485
BsaJI	3	182, 603, 2826
Bsp1286I	5	52, 761, 1922, 2007, 2414
BspHI	2	1163, 2171
BssSI	2	616, 2000
BstOI	5	183, 471, 592, 605, 2827
BstZI	1	2887
DdeI	4	718, 1127, 1293, 1833
DraI	3	1202, 1221, 1913, 2488
DraIII	1	2488
DrdI	2	551, 2532
EaeI	5	83, 282, 1724, 2860, 2887
EarI	3	327, 2131, 2768
EclHKI	1	1336
EcoICRI	1	50

Enzyme	# of Sites	Location
EcoRI	1	36
FokI	4	1302, 1483, 1770, 2806
FspI	2	1558, 2730
HaeII	4	321, 691, 2330, 2338
HgaI	4	554, 1132, 1862, 2263
HincII	1	44
HindIII	1	18
Hsp92I	1	1873
NaeI	1	2382
NciI	3	823, 1519, 1870
NgoMIV	1	2380
NotI	1	2887
NsiI	1	9
NspI	1	447
PvuI	2	1706, 2751
PvuII	2	267, 2780
RsaI	1	1816
SacI	1	52
SalI	1	42
ScaI	1	1816
SfaNI	5	16, 540, 1592, 1783, 2032
SfiI	1	82
SinI	2	1474, 1696
SpeI	1	12
SspI	2	2140, 2693
TaqI	4	43, 543, 1987, 2450

Table 1. Restriction Enzymes That Cut the pGEM®-9Zf(–) Vector Between 1 and 5 Times. (continued)

Enzyme	# of Sites	Location
TfiI	2	278, 418
Tth111I	1	2879
VspI	3	214, 273,1508
XbaI	1	27
XmnI	1	1935

Table 2. Restriction Enzymes That Do Not Cut the pGEM®-9Zf(–) Vector.

AatII	BclI	Csp45I	NdeI	SnaBI
Acc65I	BglII	CspI	NheI	SphI
AccB7I	BlpI	Eco47III	NruI	StuI
AccIII	BsaBI	EcoNI	PacI	StyI
AgeI	BsaMI	EcoRV	PmeI	SwaI
ApaI	BspMI	FseI	PmlI	XcmI
AscI	BsrGI	HpaI	PpuMI	XhoI
AvaI	BssHII	I-PpoI	PshAI	XmaI
AvrII	Bst98I	KasI	PstI	
BamHI	BstEII	KpnI	SacII	
BbeI	BstXI	MluI	SgfI	
BbsI	Bsu36I	NarI	SgrAI	
BbuI	ClaI	NcoI	SmaI	

Table 3. Restriction Enzymes That Cut the pGEM®-9Zf(–) Vector 6 or More Times.

AciI	DpnI	HphI	MspA1I	Sau96I
AluI	Fnu4HI	Hsp92II	MspI	ScrFI
BbvI	HaeIII	MaeIII	NdeII	Tru9I
BsrSI	HhaI	MboI	NlaIV	XhoII
BstUI	HinfI	MboII	PleI	
CfoI	HpaII	MnlI	Sau3AI	

5. Related Products

pGEM® Vectors

Product	Size	Cat. #
pGEM®-3Z Vector	20µg	P2151
pGEM®-4Z Vector	20µg	P2161
pGEM®-3Zf(+) Vector	20µg	P2271
pGEM®-3Zf(–) Vector	20µg	P2261
pGEM®-5Zf(+) Vector	20µg	P2241
pGEM®-5Zf(–) Vector	20µg	P2351
pGEM®-7Zf(+) Vector	20µg	P2251
pGEM®-7Zf(–) Vector	20µg	P2371
pGEM®-11Zf(+) Vector	20µg	P2411
pGEM®-13Zf(+) Vector	20µg	P2541

All pGEM® Vectors are provided with a glycerol stock of bacterial strain JM109. The JM109 cells do not contain the vector and are not competent.

Other Vectors

Product	Size	Cat.#
pSP64 Poly(A) Vector	20µg	P1241
pSP72 Vector	20µg	P2191
pSP73 Vector	20µg	P2221

Sequencing Primers

Product	Size	Cat.#
SP6 Promoter Primer	2µg	Q5011
T7 Promoter Primer	2µg	Q5021